WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 11, 1662-1669 Research Article ISSN 2278 – 4357

# EXTRACTION AND QUANTIFICATION OF B-SITOSTEROL FROM SIDA ACUTA

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Article Received on
18 September 2018,

Revised on 08 October 2018,
Accepted on 29 Oct. 2018,

DOI: 10.20959/wjpps201818-12708

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## ABSTRACT

In the today's modern era, herbal medicines are used for primary health care due to better acceptability by the human body with lower level of side effects in medical treatment. The activity and efficacy shown by many herbal medicines can be measured by sophisticated techniques like HPTLC. The correct botanical identity also helps in regulating the chemical sanctity of the herbs. The present work reports the method of extraction and quantification of Beta-Sitosterol from *Sida Acuta*. Beta-Sitosterol is found in plant like *Sida Acuta* commonly known as Bala. The samples of *Sida Acuta* were extracted by using HPLC grade methanol solvent in Soxhlet apparatus. A solution of concentration 250 mg/ml was prepared for phytochemical analysis of the extract. HPTLC method was used for the quantification of Beta-Sitosterol in the sample. The samples were sonicated for 10 mins dissolution. 1mg/ml *Sida Acuta* and 0.1mg/ml Beta-Sitosterol samples for the experiment. A total of 10 spots were applied on to the plate using Linomat 5 applicator. The solvent system used was Toluene: Ethyl Acetate: Glacial Acetic Acid (8:2:0.2) was used. The samples were derivatized using Anisaldehyde-Sulphuric Acid Reagent (ASR Reagent) and the plate was scanned at 580nm wavelength. From the standard graph the amount of Beta-Sitosterol present in the sample was calculated.

**KEYWORDS:** Herbal Medicine, HPTLC, Sida Acuta, β-Sitosterol.

## INTRODUCTION

Traditional system of medicine like Ayurveda continues to be widely practiced in India. It is estimated that 80% of the population uses traditional drugs. There has been a steep increase in the interest in traditional forms of medicine recently. The reason is disillusionment due to

harmful side effects of modern drugs, unsatisfactory treatment of many diseases and conditions such as allergy, arthritis, hepatic disorders, obesity, degenerative diseases and mental stress, etc. and in case of terminal illness, the desire to try every possible treatment including alternative forms of healing. The WHO estimates that 80% of the people in the developing countries of the world rely on traditional medicines for their healthcare needs and about 85% of the traditional medicines involve the use of plant extracts.

Plants have a long history of acting as tonics, restoratives, medicines and also as poisons. The reason for the use of herbs in treating the sick and the afflicted has survived for thousands of years. In the today's modern era, herbal medicines are used for primary health care due to better acceptability by the human body with lower level of side effects in medical treatment, these medicines are organic in nature and source of all life on earth. Evaluation of plant materials and their derived products has always been an important part of the analyst. However, over the years the nature and degree of this evaluation has changed. Initially, it was considered sufficient to authenticate the plant material by comparison with standard botanical description or a monograph. Later, it was realized that for determination of adulterants, the above data must be supplemented with both, microscopical analysis and confirmatory chemical tests for the constituents present. This development process continued slowly until the middle of the last century when rapid advances in the knowledge of chemistry of plant drugs were made and new, improved methods for the analysis, identification and estimation of the active ingredients were developed. This led to the requirement that drugs should conform to a phytochemical as well as morphological monograph.

Simultaneous processing of sample and standard under similar condition gives better analytical precision and accuracy through HPTLC. Additionally, extreme flexibility for selection of stationary phase, mobile phase, developing technique, detection with or without pre or post column derivatization etc are some of the advantages of HPTLC over other chromatography techniques.

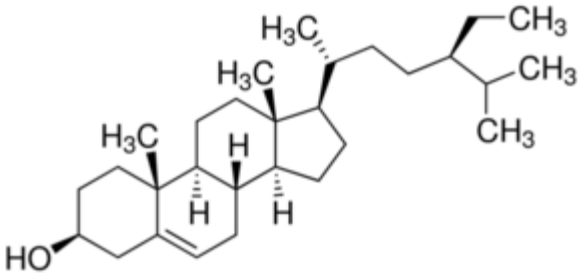
For herbal plants like *Sida Acuta* containing complex mixture of many chemical constituents, HPTLC being the open system is better for qualitative as well as quantitative analysis. In the present work like β-Sitosterol from *Sida Acuta* is analyzed using HPTLC technique.

**Sida Acuta Burm.f**

Sida acuta, the common wireweed, is species of flowering plant in the mallow family, Malvaceae.

The plants for the present research work were collected from Bhandup and Vikhroli village area in Mumbai, India. The collected plants were dried, the stem and leaves were separately crushed and the powder obtained was used from test sample preparation.

β-Sitosterol are white, waxy powders with a characteristic odour. They are hydrophobic and soluble in alcohols. β-Sitosterol is one of several phytosterols (plant sterols) with chemical structures similar to that of cholesterol.




Synonyms: Cupreol, Cinchol, α-phytosterol, Rhamnol, Quebrachol

Molecular formula: $C_{29}H_{50}O$

Molecular weight: 414.71

Chemical class/group: Terpenes (Subclass: Triterpenes)

β-Sitosterol is a main phytosterol, found in numerous plants including rice, wheat, corn, nut, peanut etc. β-Sitosterol has recorded an amazing health benefits as an hepatoprotective, antioxidant and antipyretic , inflammatory disorders and immunomodulatory , anti-inflammatory and rheumatoid arthritis. As the structure of β-Sitosterol is similar to cholesterol, it takes the place of dietary and biliary cholesterol in micelles produced in the intestinal lumens. Thus reducing the cholesterol absorption in the body.

**METHOD AND TECHNIQUE**

In this present work, an attempt has been made to develop a method for identification and quantification of β-Sitosterol. HPTLC technique is one of the recent and most advanced technique in Planar Chromatography. The technique which has been applied in many trace analysis has an entire concept that includes standardized methodology which is based on scientific facts and validated methods for quantitative and qualitative analysis. Sophisticated

instruments, controlled by an integrated software platform ensure to the highest degree of usefulness, reliability, and reproducibility of generated data.

## 1. Quantification of β-Sitosterol in *Sida Acuta* by High Performance Thin Layer Chromatography

### A. Scope

This method describes about the determination and quantification of β-Sitosterol in the herbal plant *Sida Acuta* through HPTLC Method.

### B. Principle

The samples are extracted and analyzed on HPTLC Plate Silica Gel $60F_{254}$. The Detection and quantification were performed by densitometry at λ=580nm in visible mode. Evaluation was carried out by comparing peak areas with linear regression.

### C. Equipment

Camag HPTLC instruments with Server Visions CAT software 2.4.17.

Camag Linomat 5 applicator

Camag Twin trough glass chamber

Camag TLC Scanner IV

General Laboratory Glassware.

### D. Reagents

Methanol- HPLC Grade (Merck)

Toluene, Ethyl Acetate, Glacial Acetic Acid- HPLC Grade (Merck)

Anisaldehyde-Sulphuric Acid Reagent (Merck)

β- Sitosterol analytical standards (Sigma Aldrich)

### E. Plant Preparation

The plants for the present research work were collected from the natural habitat of Bhandup and Vikhroli village area in Mumbai, India. The plants were authenticated at Blatter's herbarium; St. Xavier's College, Mumbai and the specimens voucher were deposited in the St. Xavier's College Herbarium for further reference. The accession number for Sida Acuta Burm. F. is 13836 of E. Blatter and NYD 5075 of N.Y. Das.

The collected plants were dried, the stem and leaves were separately crushed and the powder obtained was used from test sample preparation.

### F. Sample Preparation

The samples of *Sida Acuta* were extracted by using HPLC grade methanol solvent in Soxhlet apparatus. A solution of concentration 250 mg/ml was prepared for phytochemical analysis of the extract. The samples were sonicated for 10 mins dissolution. 1mg/ml *Sida Acuta* and 0.1mg/ml β -Sitosterol samples for the experiment.

Samples we marked as follows:

1. Sida Acuta Stem B1-Extract of Stem for the samples collected from Bhandup
2. Sida Acuta Leaves B1-Extract of Leaves for the samples collected from Bhandup
3. Sida Acuta Stem V2-Extract of Stem for the samples collected from Vikhroli
4. Sida Acuta Leaves V2-Extract of Leaves for the samples collected from Vikhroli.

### G. Derivitization

β- Sitosterol was not detectable in UV light or fluorescence therefore it has been transformed in detectable substances in order to evaluate the TLC separation. Derivatization of the HPTLC plate was done using Anisaldehyde-Sulphuric Acid Reagent (ASR Reagent) and the plate was scanned at 580nm wavelength. From the standard graph the amount of β -Sitosterol present in the sample was calculated.

### H. Chromatographic Condition

| Sr. No | Parameters | Description |
|---|---|---|
| 1 | Instrument | Camag HPTLC instruments with Server Visions CAT software 2.4.17 |
| 2 | Stationary Phase | Silica gel $60F_{254}$ pre-coated on aluminum sheet. |
| 3 | Mobile Phase | Toluene: Ethyl Acetate: Glacial Acetic Acid (80:20:2 v/v/v) |
| 4 | Plate Format | 200.0 X 100.0 mm |
| 5 | Application | Position Y: 8.0mm, length 8.0mm width 0.0mm |
| 6 | Pre washing of Chromatographic Plate | Methanol and activated at $110^{o}C$ for half an hour. |
| 7 | Spray Gas | Air |
| 8 | Sample Solvent Type | Methanol |
| 9 | Filling speed | 15 µL/s |
| 10 | Dosage speed | 150 nL/s |
| 11 | Band length | 8 mm |
| 12 | Development distance | 80 mm |
| 13 | Derivatizing reagent | Anisaldehyde sulphuric acid |
| 14 | Drying of plate | At Room Temperature for 5 min |
| 15 | Wavelength | Tungsten Lamp - 580 nm |

| | | |
|---|---|---|
| | | Mercury Lamp - 366 nm<br>Deuterium Lamp - 254 nm |
| 16 | Saturation Time | mins |

**I. Sample Injections Sequence, Rf values and Peak Areas corresponding to the serial dilutions of standard compound-β-Sitosterol and extract from stem and leaves**

| Sample Number | Sample Description | Type | Injection Volume | $R_{f(max)}$ | Peak Area |
|---|---|---|---|---|---|
| 1 | Sida Acuta Stem B1 | Sample | 15.0 µl | 0.526 | 0.00230 |
| 2 | Sida Acuta Stem B1 | Sample | 15.0 µl | 0.521 | 0.00290 |
| 3 | β- Sitosterol-Std | Reference | 0.5 µl | 0.517 | 0.00054 |
| 4 | β- Sitosterol-Std | Reference | 2.5 µl | 0.514 | 0.00237 |
| 5 | Sida Acuta Leaves B1 | Sample | 15.0 µl | 0.511 | 0.00254 |
| 6 | Sida Acuta Leaves B1 | Sample | 15.0 µl | 0.507 | 0.00246 |
| 7 | β- Sitosterol-Std | Reference | 4.5 µl | 0.504 | 0.00350 |
| 8 | β- Sitosterol-Std | Reference | 6.5 µl | 0.506 | 0.00452 |
| 9 | Sida Acuta Stem V2 | Sample | 15.0 µl | 0.507 | 0.00165 |
| 10 | Sida Acuta Stem V2 | Sample | 15.0 µl | 0.507 | 0.00170 |
| 11 | β- Sitosterol-Std | Reference | 8.5 µl | 0.508 | 0.00579 |
| 12 | β- Sitosterol-Std | Reference | 10.5 µl | | |
| 13 | Sida Acuta Leaves V2 | Sample | 15.0 µl | 0.510 | 0.00165 |
| 14 | Sida Acuta Leaves V2 | Sample | 15.0 µl | 0.512 | 0.00162 |
| 15 | β- Sitosterol-Std | Reference | 12.5 µl | | |

**J. Chromatograms**

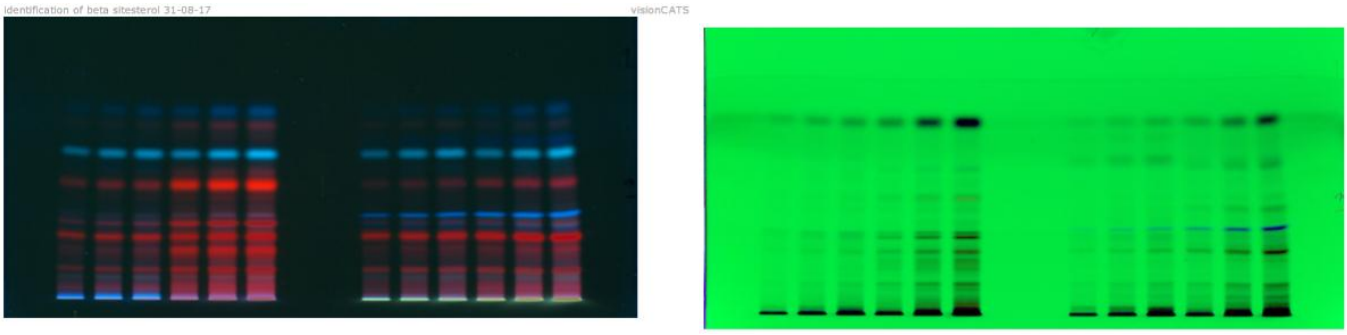



**Plate 1 and 2: HPTLC Profile of Quantification of β –Sitosterol at 366nm and 254 nm.**

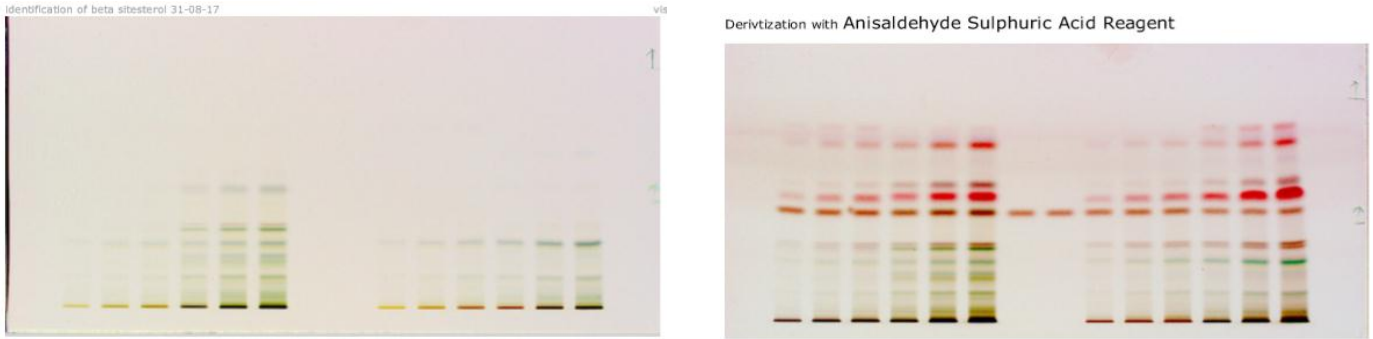
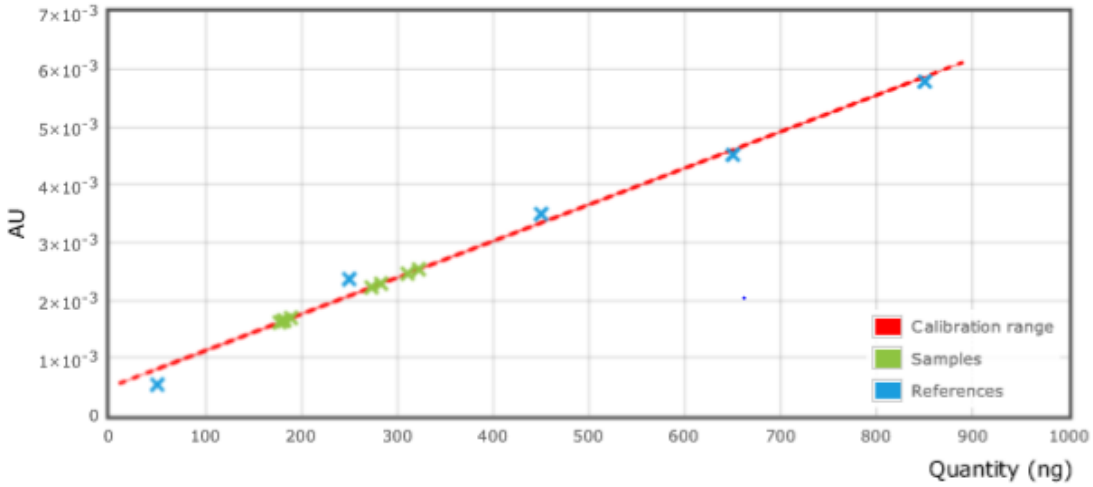



**Plate 3 and 4: HPTLC Profile of Quantification of derivatized product at 580 nm.**

| **Calibration Results:** |
|---|
| Area Calibration for substance β-Sitosterol at 580nm |




| | |
|---|---|
| Regression Mode | Linear-2 |
| Range Deviation | 5.00% |
| Related Substance | Default |
| Number of Reference | 5 |
| Calibration Function | $y = 6.325 \times 10^{-9} x + 4.983 \times 10^{-4}$ |
| Coefficient of Variation | CV 5.9197 % |
| Correlation Coefficient | R = 99.393169 % |

## K. RESULTS AND DISCUSSION

Characterization of Sida Acuta for β-Sitosterol by technique like HPTLC, methods described are simple, routine and easy to carry out. The linearity values are calculated for each technique. It gives a confirmation and sensitivity to the method.

| **Final Results:** β-Sitosterol (Quantification) | | | | |
|---|---|---|---|---|
| **Wavelength:** | 580nm | (8 Samples assigned) | | |
| **Sample 1 (Sida Acuta Stem B1)** | 18.55 µg/ml | CV = 2.529 % | (2 Applications) | 92.73 µg in 500.00 mg |
| **Sample 2 (Sida Acuta Leaves B1)** | 21.11 µg/ml | CV = 2.614 % | (2 Applications) | 105.6 µg in 500.00 mg |
| **Sample 3 (Sida Acuta Stem V2)** | 12.36 µg/ml | CV = 2.994 % | (2 Applications) | 61.78 µg in 500.00 mg |
| **Sample 4 (Sida Acuta Leaves V2)** | 12.00 µg/ml | CV = 1.988 % | (2 Applications) | 60.00 µg in 500.00 mg |

## L. CONCLUSION

It was observed that the content of β-Sitosterol was comparatively high in Sample 2 which was extracted from the leave of Sida Acuta plant collected from Bhandup Location. Characterization of Sida Acuta for β-Sitosterol by methods like HPTLC can be one of the

accurate and precise technique with an acceptable level of sensitivity with recovery of 91.87% and reproducibility C.V= 2.044% Average 0.047 (peak height).

**M. ACKNOWLEDGEMENTS**

We would like to give our sincere thanks to Anchrom Enterprises (I) Pvt. Ltd, Mulund, Mumbai and Blatter Herbarium, St. Xavier's College, Mumbai for their valuable support in this research work.

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